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β-Cell Failure in Type 2 Diabetes: A Case of Asking Too Much of Too Few?

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The islet in type 2 diabetes (T2DM) is characterized by a deficit in β-cells, increased β-cell apoptosis, and extracellular amyloid deposits derived from islet amyloid polypeptide (IAPP). In the absence of longitudinal studies, it is unknown if the low β-cell mass in T2DM precedes diabetes onset (is a risk factor for diabetes) or develops as a consequence of the disease process. Although insulin resistance is a risk factor for T2DM, most individuals who are insulin resistant do not develop diabetes. By inference, an increased β-cell workload results in T2DM in some but not all individuals. We propose that the extent of the β-cell mass that develops during childhood may underlie subsequent successful or failed adaptation to insulin resistance in later life. We propose that a low innate β-cell mass in the face of subsequent insulin resistance may expose β-cells to a burden of insulin and IAPP biosynthetic demand that exceeds the cellular capacity for protein folding and trafficking. If this threshold is crossed, intracellular toxic IAPP membrane permeant oligomers (cylindrins) may form, compromising β-cell function and inducing β-cell apoptosis. ***Diabetes*** **62:327–335, 2013**

ISLET PATHOLOGY IN TYPE 2 DIABETES

In type 2 diabetes (T2DM), the islet is characterized by a deficit in β-cells, increased β-cell apoptosis, and extracellular amyloid deposits derived from IAPP (1,2). The question has long been posed, is islet amyloid (Fig. 1) in T2DM the blood or the bullet (3,4)? In the neurosciences, the bullet hypothesis gained ascendancy under the moniker of the amyloid hypothesis in relation to Alzheimer's disease (5). Arguably, the diabetes field was appropriately more skeptical because evidence in favor of a direct toxic effect of islet amyloid (6) was outnumbered by studies that did not identify such toxicity (7,8). However recent progress has seen a convergence of ideas by those pursuing insights into the possible link between protein misfolding and cellular degeneration in the neurosciences and the islet field. The emerging alternative but related toxic oligomer hypothesis can be summarized as follows.

Amyloid deposits occur as a consequence of misfolding and mistrafficking of proteins with the propensity to form amyloid deposits. These proteins may form a variety of oligomers, the most toxic of which are those that form relatively early and form in or interact with cellular membranes (7,9). In contrast, if misfolded IAPP oligomers organize into amyloid fibrils, these are generally less toxic but also relatively inert and as such tend to accumulate in the extracellular space where they may play a role as a physical barrier and as such contribute to cellular dysfunction (4,10). In order to appreciate why some proteins have the propensity to form oligomers and amyloid fibrils, it is helpful to consider the physical interactions that these proteins share in common.

PROTEIN MISFOLDING AND OLIGOMERIZATION

A common feature of amyloid proteins, including IAPP, is the ability to misfold into highly polymorphic oligomeric and fibrillar structures. In vitro experiments have shown that oligomers appear early during the misfolding process, whereas fibrils represent the end point of misfolding (11,12). Although some oligomers are likely to be on pathway to fibril formation, others are not (Fig. 2). IAPP fibrils exhibit the classical cross-β structure typical for amyloid fibrils (13), and structural models have been obtained using a number of experimental methods (14–16). Best understood structurally are IAPP fibrils with striated ribbon and twisted morphology (17), for which detailed three-dimensional structural information has been obtained (Fig. 2) (14,15). In both cases, IAPP takes up two-stranded structures. The main difference lies in how the two strands are arranged with respect to each other (Fig. 2). In the model from striated ribbon fibrils, the strands are approximately in the same plane, whereas the strands from twisted fibrils are more staggered. As is typical for almost all disease-related amyloid proteins (18), both IAPP fibril types take up a parallel and in-register structure. In these structures, the same residues from different molecules stack on top of one another. This structure is stabilized by the stacking of the same hydrophobic residues, which is much more favorable than that of residues with like charges (18). Oligomer formation is also facilitated by the interaction of hydrophobic residues, but much less is known about oligomer structures in general. This is likely due to their often transient and polymorphic nature, which makes it more difficult to study them using structural methods (19). Nevertheless, it is clear that a large range of oligomers of varying sizes and structures exist and that they share the general property of cytotoxicity (19–21). Different conformationally specific antibodies have been used to recognize different oligomer types (20,22–24). Remarkably, some of these antibodies can detect oligomers from a wider range of amyloid proteins, including IAPP. A11 is a conformationally specific antibody that recognizes a subset of toxic oligomers from a wide range of amyloid diseases (20). Although no direct structural information is available for any disease-causing amyloid oligomer, Eisenberg and colleagues (25) recently reported a major advance. They were able to determine the crystal

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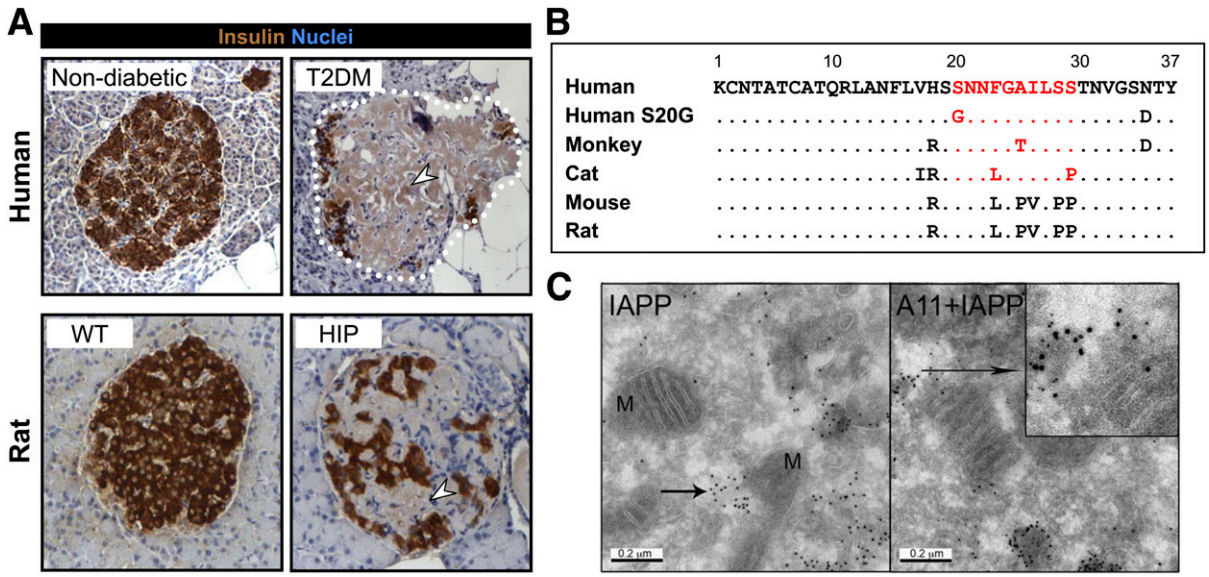

FIG. 1. ***A*****: Human islets from a nondiabetic subject and a subject with T2DM (*upper panel*) and from a wild-type (WT) and a human IAPP transgenic (HIP) rat (*lower panel*) stained for insulin (brown). Deposits of amyloid derived from IAPP are indicated by a white arrowhead. Original magnification: ×40.** ***B*****: Alignment of IAPP ortholog proteins. Amino acid alignment of IAPP protein sequences identified in Homo sapiens (human, CAA39504), human mutant (S20G), Macaca mulatto (monkey, XP_001098290), Felis catus (cat, NP_001036803), Mus musculus (mouse, NP_034621), and Rattus norvegicus (rat, NP_036718). Dots correspond to conserved residues with human IAPP sequence. Red letters correspond to the amyloidogenic sequence.** ***C*****: Sections of islets from human IAPP transgenic mice labeled for oligomers (A11) and IAPP (5 nm and 10 nm gold, respectively). IAPP- and oligomer-labeled aggregates were found adjacent to mitochondria (M), and mitochondrial integrity appeared to be compromised (black arrow points to the aggregates penetrating mitochondria). Original magnification: ×120,000. This figure originally appeared in an article by Gurlo et al. (50).**

structure of A11-positive oligomers derived from a short αB-crystallin peptide fragment. As with amyloid oligomers in general, oligomers of this short peptide fragment were toxic. In the crystal structure, this peptide took on an overall cylindrical shape with antiparallel strands, which the authors termed cylindrin (Fig. 2). This study could provide important insights into the structure of other A11-positive oligomers.

Based on the existing structural information available for IAPP fibrils and oligomers, it is clear that IAPP is a typical amyloid protein, which likely exerts its toxicities via the same mechanisms as other amyloid proteins. This similarity also includes membrane interaction. Membranes play an important dual role in the misfolding of amyloid proteins. Membranes are not only disrupted by the toxic action of misfolded oligomers, but they can also accelerate oligomer and fibril formation by orders of magnitude (26). In the case of IAPP, it was found that this acceleration is mediated by an α-helical intermediate that forms in the presence of negatively charged lipids (27–29). A lipid that can potently activate this misfolding pathway is phosphatidyl serine, which is commonly found in the cytosolic leaflets of cellular membranes. Thus, IAPP molecules that escape the secretory pathway into the cytoplasmic space are likely to rapidly misfold and disrupt membrane integrity in vivo.

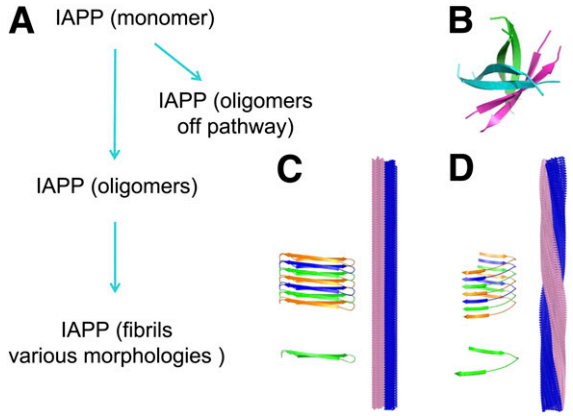

FIG. 2. IAPP misfolding pathways. ***A*****: Schematic illustration of a stepwise misfolding pathway of IAPP that generates toxic oligomers as well as a range of different fibril types. Although the structure of IAPP oligomers remains elusive, the crystal structure of an A11 antibody–positive oligomer structure has recently been reported (25) and is shown in panel** ***B*****.** ***C*** **and** ***D*****: The reported structures for fibrils with striated ribbons and twisted morphologies. The bottom shows the structure of single IAPP molecule (green). In case of the striated ribbon, the two strands are approximately in the same plane while the two strands in the fibrils with twisted morphology are offset. Individual stacks of monomers were built using MFIBRIL (http://chemsoft.hsc.usc.edu:8080/MFIBRIL/) and colored green, brown, and blue. MFIBRIL was then used to dock individual stacks together to better mimic the fibril structure (blue and magenta). Note that contacts in the striated ribbons are made between same monomeric subunits, whereas contacts in the twisted fibrils are more staggered by packing strands from one monomer subunit against strands from a monomer three layers above.**

DEFENSES AGAINST PROTEIN MISFOLDING AND AGGREGATION

The workload of a typical β-cell in protein synthesis, folding, sorting, processing, and then disposal by either secretion or degradation is remarkable (Table 1). An average human β-cell synthesizes, folds, processes, and then either secretes or degrades ~10,000 proinsulin molecules per minute even in the basal nonfed state (30,31). In addition, each β-cell synthesizes, folds, processes, and then either secretes or degrades ~1,000 pro-IAPP molecules per minute (32).

IAPP is assembled in the endoplasmic reticulum (ER) as prepro-IAPP, an 89–amino acid protein, and then

TABLE 1
Estimated number of insulin molecules secreted per minute by a typical β-cell in a lean individual in the fasting state

A lean individual (70 kg) secretes 2 pmol/kg/min insulin in fasting state	
Insulin (mol/min)	$\sim 140 \times 10^{-12}$
Insulin (molecules/min)*	$\sim 84 \times 10^{12}$
Insulin (molecules/min/β-cell)†	~10,500

Given that ~30% proinsulin fails quality control, that only a relatively small amount of insulin synthesized is secreted, and that half the 24-h cycle is in the fed state, the likely synthetic rate of proinsulin per β-cell per minute is likely to be much higher in reality. The estimations take into account the following: *$\sim 6 \times 10^{23}$ molecules in one mole. †$\sim 800 \times 10^{6}$ β-cells in an adult human.

processed to its mature 37–amino acid form within the secretory pathway (Fig. 3) (33). The microenvironment of the ER favors appropriate folding and maturation of ER proteins, avoiding protein aggregation (34,35). The rate of synthesis and delivery of proinsulin and pro-IAPP into the ER is adaptively constrained to the rate at which the ER can successfully fold and export these proteins into the secretory pathway by a feedback signaling system termed the unfolded protein response (34,35). The microenvironment and chaperones in β-cell secretory vesicles are also protective against IAPP aggregation (36–43). Proteins that fail the ER quality control system are removed from the ER and degraded by endoplasmic reticulum-associated degradation (ERAD), also known as the ubiquitin/proteasome system (44). Misfolded proteins are translocated to the cytosol and ubiquinated. Polyubiquinated proteins are then deubiquitinated prior to passage through the proteasome (45). If misfolded proteins form aggregates, they are removed by macroautophagy (hereafter referred to as autophagy) (46,47). An isolation double membrane forms in the cytoplasm to surround such intracellular targets to generate an autophagosome that then fuses with a lysosome in which the sequestered material is degraded by hydrolytic enzymes (Fig. 3). The autophagy/lysosomal pathway is required for survival and function of β-cells and is adaptively increased under conditions of increased β-cell protein synthesis, for example in obesity (48,49). The autophagy/lysosomal pathway is particularly important for protection of long-lived cells against accumulation

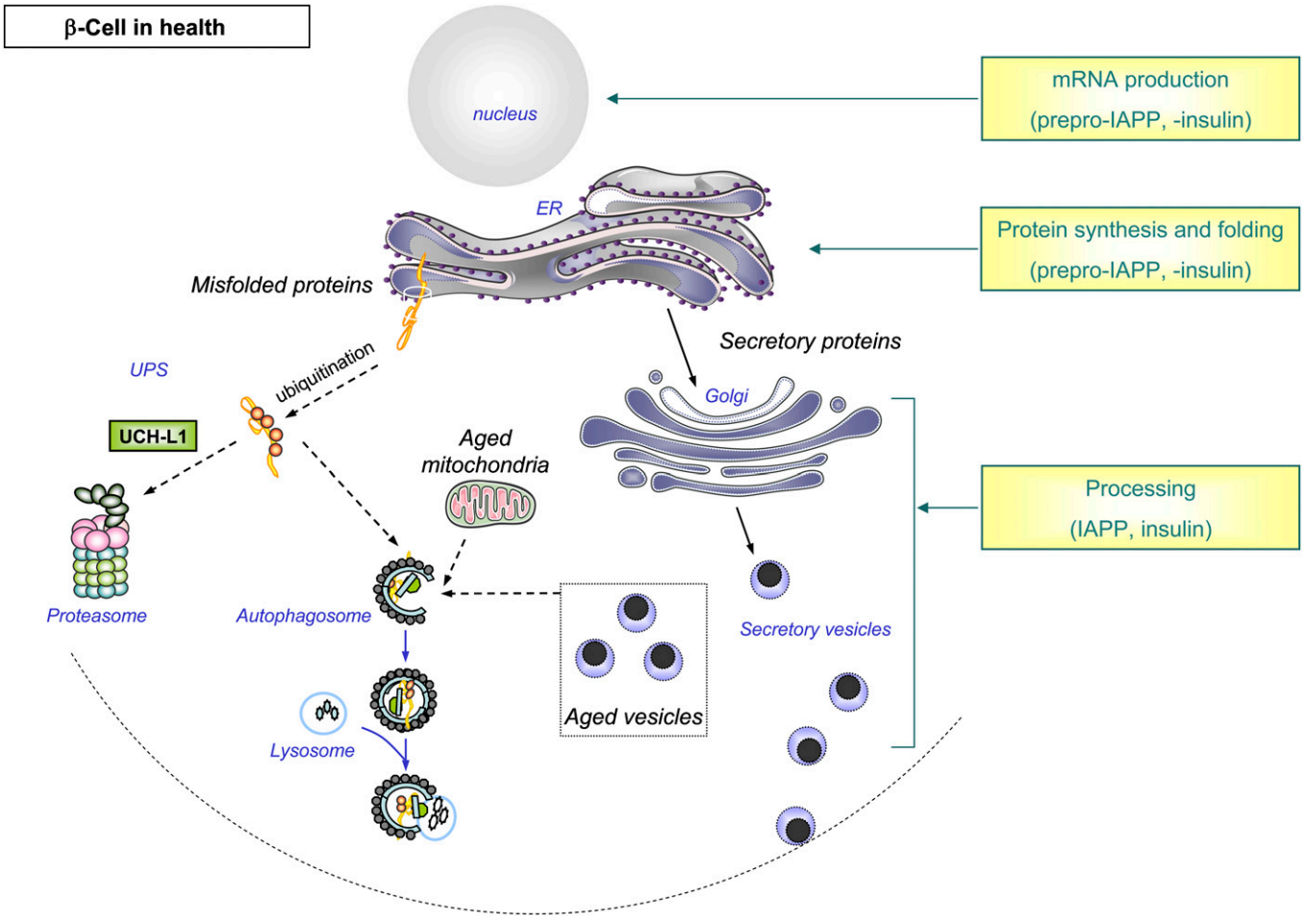

FIG. 3. Secretory pathway and mechanisms of β-cell defense against protein misfolding. The major β-cell secretory proteins, insulin and IAPP, are synthesized and folded in the ER and then processed within the secretory pathway (Golgi and secretory vesicles). Misfolded proteins are targeted to the ER-associated degradation, also known as ubiquitin-proteasome system (UPS), that involves ubiquitination of the targeted proteins, their deubiquitination by enzymes such as UCH-L1, and subsequent degradation by the proteasome. If the ubiquitin-proteasome system fails or if protein aggregates form, an alternative pathway of protein clearance becomes available: the autophagy pathway in which membranes surround the material to be degraded (ubiquitinated proteins and protein aggregates but also damaged organelles and aged vesicles) to form autophagosomes that fuse with lysosomes to allow degradation of their content.

of toxic amyloidogenic oligomers such as IAPP and Alzheimer's β-protein (49–51).

Given the extraordinary workload of a typical β-cell, the high potency of human IAPP to form toxic oligomers, and the long lifespan of β-cells in humans (52), it is a remarkable tribute to the defenses against protein misfolding and aggregation that even in the setting of the further increased workload in obesity, most β-cells do not accumulate sufficient toxic oligomers to compromise β-cell function in most individuals.

DEFENSES AGAINST PROTEIN AGGREGATION OVERCOME: THE THRESHOLD CONCEPT

In humans with T2DM, the formation of intracellular oligomers and extracellular amyloid fibrils implies that the mechanisms to prevent accumulation of misfolded proteins are overcome (50). There is a threshold of IAPP expression that if exceeded leads to formation of IAPP oligomers and the adverse consequences that accrue (53–57). This threshold may be breached because the burden of protein synthesis is increased to a level that exceeds the capacity of a healthy β-cell to fold, process, and dispose of the proteins (by secretion or degradation) and/or because the threshold is decreased. Human IAPP transgenic rodent models and transduced β-cells imply that both of these contribute (53–57). Human IAPP transgenic mice and rats develop diabetes in a transgene dose-response manner (55), or if human IAPP expression is increased by drug- or obesity-induced insulin resistance (56,57).

However once the threshold for successful protein synthesis and disposal is overcome, the ubiquitin/proteasome and autophagy/lysosomal systems for elimination of protein aggregates become defective, further compromising the capacity for protecting the cell from formation of toxic IAPP oligomers (vide infra) (49,58).

THRESHOLD FOR SUCCESSFUL PROTEIN FOLDING AND DISPOSAL OVERCOME: A ROLE FOR β-CELL MASS FORMED IN CHILDHOOD?

The most common risk factor for T2DM is obesity. With increasing obesity (BMI), insulin resistance increases, requiring increased expression of insulin and IAPP (59). To appreciate the synthetic workload placed on β-cells in an individual, it is necessary to consider not only the overall insulin demand but also the number of β-cells by which this demand is met in that individual. The number of β-cells in humans increases during early childhood through the mechanism of β-cell replication and then remains relatively constant through adult life once the capacity for β-cell replication declines after early childhood (1,2,60). An underappreciated but potentially important characteristic of the period of β-cell mass expansion during the postnatal period is the wide range of β-cell mass that then accrues (Fig. 4). A wide variance in pancreatic β-cell fractional area and/or mass has been observed in adult humans, monkeys, pigs, and rodents (1,60–64). This variance is likely due in part to the intrauterine environment (65) and in part to genetic variance (vide infra) (66).

If we consider the interaction of obesity-induced insulin resistance and the wide range of β-cell mass after postnatal expansion, the increment in the protein synthetic burden per β-cell increases much more steeply in those with a low versus high number of β-cells (Fig. 5).

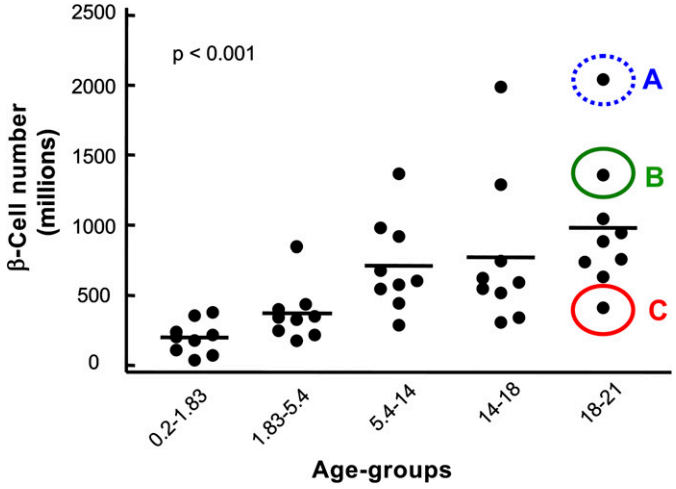

FIG. 4. β-Cell mass growth varies widely in childhood. Postnatal expansion of β-cell number plays a major role in establishing β-cell mass in adult humans and is highly variable between individuals. Data are from Meier et al. (60). Total number of β-cells in 46 children aged 2 weeks to 21 years. Data are represented as individual data points. Individuals with high (A, blue), intermediate (B, green), and low (C, red) β-cell numbers are shown for consideration of β-cell workload in response to obesity in Fig. 5.

THRESHOLD FOR SUCCESSFUL PROTEIN FOLDING AND DISPOSAL OVERCOME: A ROLE FOR ADOLESCENCE, PREGNANCY, AND AGING?

Transient insulin resistance and therefore increased β-cell workload occurs during adolescence in relation to high levels of growth hormone and sex steroid secretion (67) (Fig. 6). Obesity in adolescence superimposes additional insulin resistance and β-cell workload. We propose that in individuals with a low innate β-cell mass after childhood (individual C, Figs. 4 and 6), the β-cell workload may exceed the threshold for protein folding so that toxic IAPP

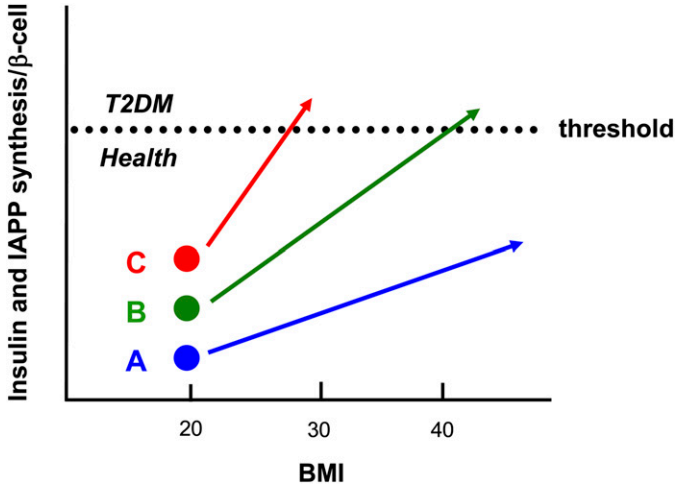

FIG. 5. Interaction of postnatal β-cell mass and BMI on insulin and IAPP synthetic demand. Schematic representation of the risk of T2DM in individuals with high (A, blue), intermediate (B, green), and low (C, red) β-cell mass formed after postnatal growth (see Fig. 4) with consideration of their BMI. The increment in the protein synthetic burden per β-cell increases more steeply in those with low (individual C) versus a high number of β-cells (individual A). The burden placed on β-cells by obesity is thus higher in individual C, as is the risk to breach the threshold for protein folding and disposal, ultimately leading to β-cell failure in T2DM.

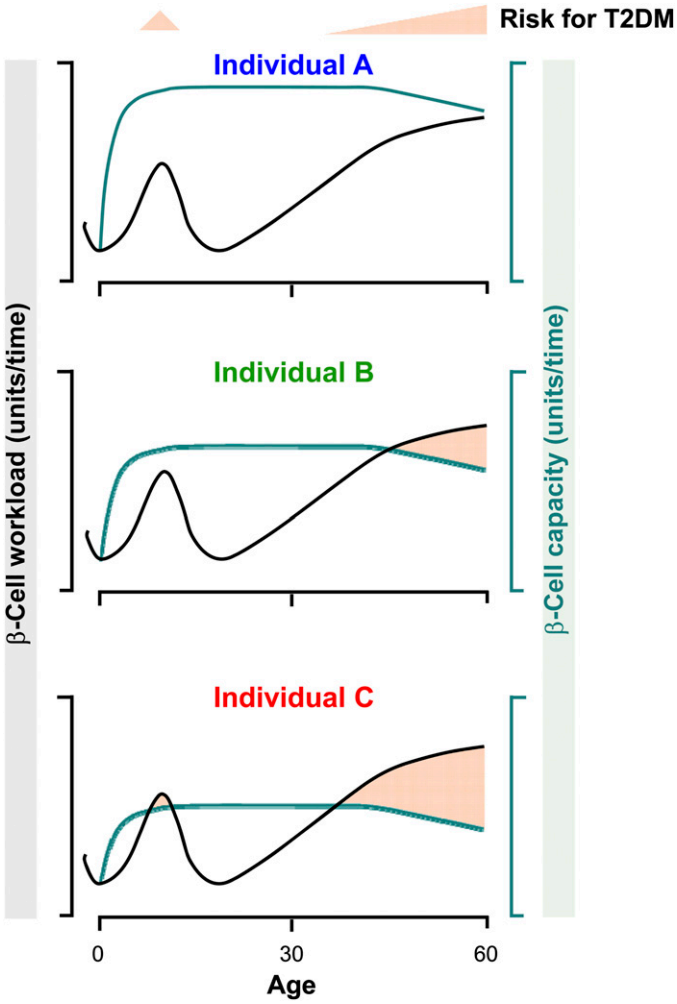

FIG. 6. β-Cell workload and risk of T2DM. Schematic representation of β-cell workload (in black) and β-cell work capacity (green) throughout life. β-Cell workload increases transiently during adolescence and progressively with aging. The capacity for β-cell workload is defined by the β-cell mass after the postnatal expansion (high in individual A, intermediate in individual B, and low in individual C, see Fig. 4) and β-cell ability to defend against protein misfolding (declines in all individuals with aging). T2DM risk increases when workload exceeds capacity (light orange), in adolescence and early adult life in C, later in B, and only with advanced age in A.

oligomers may then form, leading to β-cell dysfunction and, with time, β-cell loss and diabetes. A similar argument can be made for the transient insulin resistance in the third trimester of pregnancy leading to diabetes in some individuals. If the period of insulin resistance is rapidly reversed (as in delivery of the baby), then provided that β-cell mass has not been sufficiently degraded, reversal of diabetes may occur. Moreover, if the gestational diabetes mellitus resolves, the risk for subsequent development of T2DM depends on the β-cell workload in that individual (68).

The incidence of T2DM increases with aging (69). In most individuals, insulin sensitivity declines with aging (70). There are also age-related changes in long-lived cells, such as β-cells, that likely decrease the threshold for successful protein synthesis, folding, and disposal. Aged cells accumulate mitochondrial mutations leading to increased production of reactive oxygen species (71). The latter react with and damage proteins, placing an increased burden on the pathways for clearance of denatured proteins and damaged organelles (71). Furthermore, the mechanisms that defend against accumulation of misfolded or aggregated proteins in long-lived cells decline with aging (72). There is decreased chaperone protein availability and decreased function of the ubiquitin/proteasome system with aging (72). Acquired proteasome abnormalities such as pesticide exposure contribute to the pathogenesis of Parkinson's disease, characterized by synuclein aggregation (73). Given the parallels between neurons in neurodegenerative diseases and islets in T2DM, it would be of interest to determine if age-related changes and environmental insults/pesticides inhibit proteasomal function in β-cells and thus contribute to the pathogenesis of T2DM.

The autophagy/lysosomal pathway also declines with age (74). In liver of old rodents, fusion of autophagosomes with lysosomes is impaired (75). Several age-related brain diseases are also considered as disorders of lysosomal function, and the mechanisms of neurodegeneration are related to degradative failure and lysosomal destabilization (76). Decreased clearance of β-amyloid was reported in late-onset Alzheimer's disease (77). The autophagy/lysosomal pathway is impaired in β-cells in T2DM (78).

With the gradual increasing β-cell workload in a typical individual, the vulnerability of β-cells in that individual to cross the β-cell threshold for protein synthesis and folding will depend on the β-cell mass present at the end of childhood. Thus in individual A (Fig. 6), even in late life the relatively high β-cell mass that arose in childhood is sufficient. In individual B, with an intermediate β-cell mass, the capacity for β-cell protein folding is only overcome in late adult life with increasing insulin resistance. In contrast, in individual C, if he/she did not develop sustained diabetes due to obesity in adolescence or with pregnancies, diabetes onset is still likely at a young age. An implication of this model for T2DM is that if β-cell workload is maintained at relatively low levels by avoiding insulin resistance, then T2DM can be avoided in most individuals.

LESSONS FROM GENETICS?

Genome-wide association studies (GWASs) have revealed a variety of T2DM susceptibility genes (e.g., *KCNJ11*, *TCF7L2*, *CDKAL1*) that are mainly involved in pancreatic β-cell maturation and function (rev. in 79). Of interest, several of these genes regulate the cell cycle and therefore may play a role in the β-cell numbers that arise during the period of postnatal expansion through β-cell replication (such as *CDKAL1* and *CDC123* [80]). Among other identified genes, *WFS1* (encoding Wolframin) has an essential role in the ER unfolded-protein response and ER homeostasis (81) and is involved in granule acidification in β-cells (82). Any genetic alteration of *WFS1*, by its action to compromise ER function and intravesicular pH, might be expected to increase the risk of human IAPP misfolding and oligomerization. T2DM is also associated with gene variants associated with insulin-degrading enzyme. Insulin-degrading enzyme degrades IAPP and inhibits IAPP aggregation and toxicity in vitro (83). A rare missense mutation, S20G, that leads to increased propensity of IAPP to form oligomers is associated with T2DM, providing a proof of principal of the potential importance of IAPP in the pathogenesis of diabetes (84,85).

Although insulin resistance is a well-known risk factor for T2DM (86), GWASs have uncovered relatively few

associations with T2DM attributable to insulin-signaling pathways. However the current model links insulin resistance from any cause to T2DM risk through formation of inadequate β-cell number in childhood and/or a reduced capacity for protein folding, and both these mechanisms are prominent in the GWASs to date.

WHERE DO OLIGOMERS FORM, AND HOW DO THEY DAMAGE CELLS?

Amyloidogenic proteins such as IAPP appear to induce cytoxicity by disrupting cellular membrane integrity in the form of small nonfibrillar oligomers (7). Toxic IAPP oligomers form within the secretory pathway but are then found liberated from this compartment adjacent to disrupted vesicle membranes (Fig. 1*C*) (50). Moreover, membranes of mitochondria adjacent to these cytosolic IAPP toxic oligomers are disrupted, implying that β-cell function as well as viability is likely compromised by toxic IAPP oligomers (Fig. 1). This is supported by the decline in β-cell function that precedes loss of β-cell mass in the human IAPP transgenic rat model of T2DM (64).

It is unclear where the toxic oligomers form within the secretory pathway and even to what extent pro-IAPP has been processed before these oligomers form. Since toxic oligomers of amyloidogenic proteins form on an alternative pathway to the majority of amyloid fibrils, just because IAPP-derived amyloid is primarily composed of the 37–amino acid form of IAPP does not mean that the toxic oligomers are. A case has been made that these toxic oligomers might be composed of pro-IAPP (87). Pro-IAPP oligomers could form in the ER and/or Golgi, whereas IAPP oligomers would form in insulin secretory vesicles where pro-IAPP processing to IAPP is completed.

Toxic oligomers associated with ER membranes (50) may contribute to ER stress in β-cells of individuals with T2DM (88). Human IAPP transgenic rodent models of T2DM that form toxic IAPP oligomers have ER stress–induced apoptosis (88,89). In those models, toxic oligomers are found in association with the ER membrane, which is likely the cause of unregulated ER Ca^{2+} release to the cytosol and consequent hyperactivation of the Ca^{2+} sensitive protease calpain-2 (53). The presence of this deleterious mechanism in humans was supported by the detection of the cleaved form of α-spectrin, a marker of calpain hyperactivation, in β-cells of individuals with T2DM (53).

Once IAPP toxic oligomers are formed, they disrupt the pathways of protein clearance and likely thereby lead to further accumulation of protein aggregates. The ubiquitin/proteasome system is dysfunctional in β-cells of human individuals with T2DM, as demonstrated by the accumulation of polyubiquitinated proteins (58). Increased expression of oligomerization-prone human IAPP leads to an accumulation of polyubiquitinated proteins mediated by a deficit in the deubiquitinating enzyme ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) (58). UCH-L1 downregulation enhances ER stress–induced β-cell apoptosis, and UCH-L1 deficiency was observed in β-cells of individuals with T2DM (58). Therefore, defective protein degradation in β-cells in T2DM can, at least in part, be attributed to misfolded human IAPP leading to UCH-L1 deficiency, which in turn further compromises β-cell viability by exacerbating ER stress.

In summary, once the threshold for successful synthesis, folding, processing, and secretion of IAPP is breached and intracellular toxic oligomers begin to form, unless this is rapidly reversed, a cascade of events occurs that further compromises β-cell function and increases vulnerability to β-cell apoptosis (Fig. 7). Moreover, the resulting declining β-cell number adds an increased synthetic burden on the remaining β-cells exacerbating the accumulation of toxic oligomers.

EXTRACELLULAR ISLET AMYLOID AS A DIFFUSION BARRIER?

Although accumulating data suggest that toxic IAPP oligomers form intracellularly (50,55,90), and on a separate pathway to the majority of fibrils present extracellularly (25), this does not rule out a contributory role of extracellular islet amyloid in β-cell dysfunction. It is not known why extracellular islet amyloid forms in most islets in T2DM and occasional islets in nondiabetic individuals. The most obvious explanation is that it represents the debris from cell apoptosis trapped on the vascular endothelium where it appears to accumulate. Support for this is provided by the absence of extracellular islet amyloid in in vivo models of relatively rapid β-cell loss with high human IAPP expression (55) but accumulation of extracellular islet amyloid in more gradual-onset in vivo models (57,64).

However, islet amyloid develops rapidly in islets derived from a human IAPP transgenic mouse in vitro. Also in this model, it was reported that there was no evidence of ER stress and that toxicity was attributed to extracellular islet amyloid (91). It is difficult to interpret studies of β-cell apoptosis in isolated islets in which there is already a markedly increased frequency of β-cell apoptosis due to anoxia and nutritional deprivation of the majority of cells. In vivo, each β-cell is directly supplied by oxygen and nutrients via an afferent vascular capillary loop, whereas in isolated islets only cells at the out rim of the sphere of ~3,000 cells have direct nutrient and oxygen supply, the remainder requiring diffusion through the sphere of cells given the loss of a vascular supply. It is also therefore perhaps not surprising that there is rapid accumulation of extracellular IAPP-derived islet amyloid in vitro because there is no means to export the secreted IAPP or IAPP debris from apoptotic cells that accumulates between cells. This rapidly accumulating amyloid between cells in vitro presumably also acts as a diffusion barrier and, as such, may contribute to β-cell apoptosis in islets in vitro.

The question arises, does the extracellular islet amyloid in vivo contribute to β-cell dysfunction or apoptosis in T2DM in the vascularized islet? We have found no relationship between islet amyloid and β-cell apoptosis in humans with T2DM (1) or transgenic human IAPP rodent models (8). On the other hand, Jurgens et al. (92) report an increase in a derivative of β-cell apoptosis (β-cell apoptosis/insulin-positive area/islet area) related to a score of islet amyloid in humans with T2DM and nondiabetic individuals in the same analysis.

A more compelling case for an adverse effect of extracellular islet amyloid can perhaps be made for transplanted human islets. Extracellular islet amyloid also develops rapidly in transplanted human islets (93), a circumstance that more closely mirrors islets in vitro, since transplanted islets take several days to reestablish a vascular supply (94). During that period there is rapid loss of β-cells, presumably in part because of anoxia and nutrient deprivation but perhaps exacerbated by the diffusion barrier of extracellular islet amyloid. A case can also be made that the

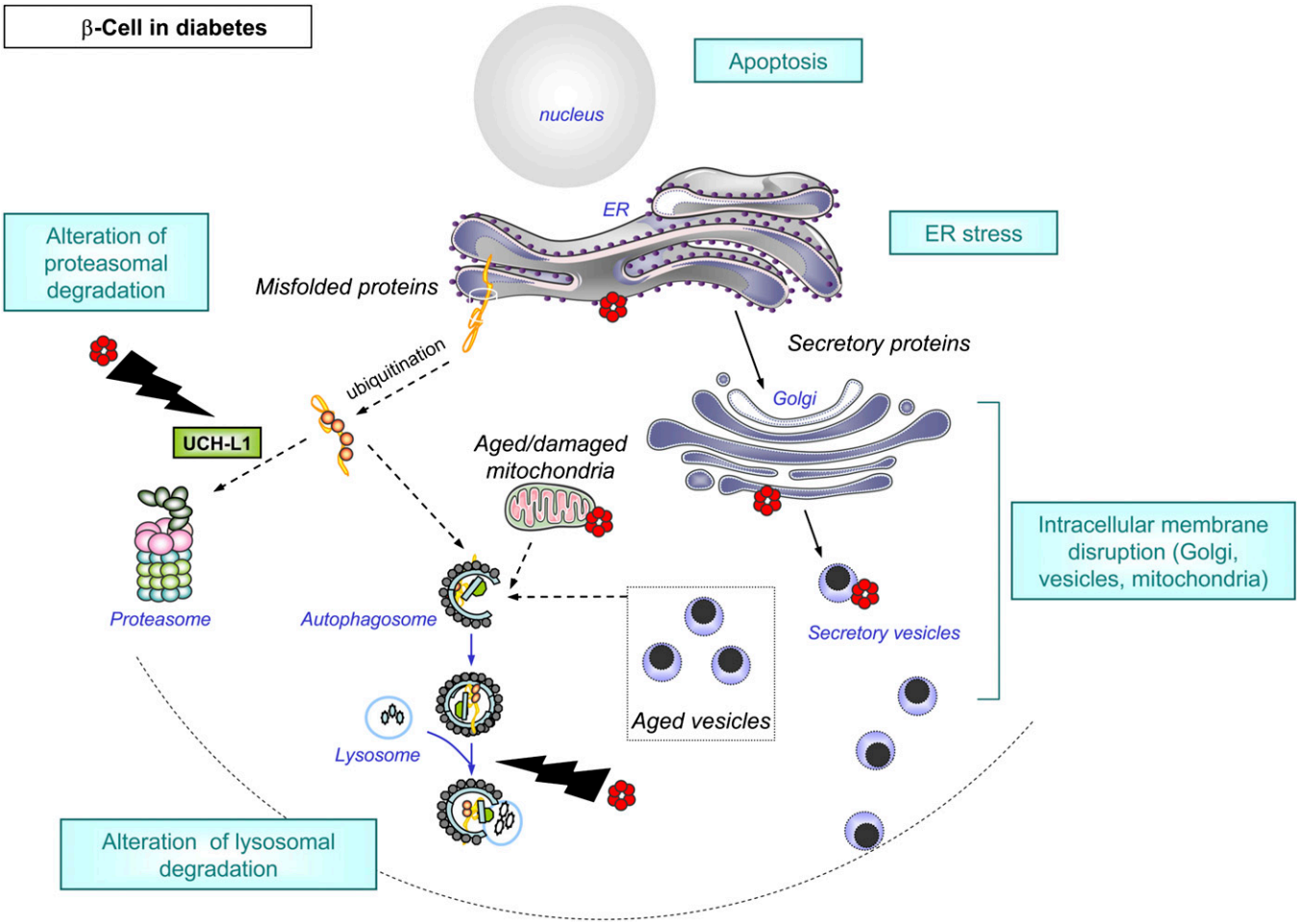

FIG. 7. Consequences of formation of intracellular toxic IAPP oligomers (cylindrins) in T2DM. Toxic IAPP oligomers (in red) are formed intracellularly in β-cells and escape from the secretory pathway leading to intracellular membrane disruption (ER, Golgi, vesicles, mitochondria), ER stress, alteration of proteasomal degradation through deficit in UCH-L1, and alteration of the autophagy/lysosomal degradation, ultimately leading to β-cell failure and apoptosis (49,50,53,58).

extracellular islet amyloid might compromise cell to cell communication, known to be important for islet function. It is unknown at present to what extent this might be relevant in vivo.

CONCLUSION

Cross-sectional autopsy studies reveal a β-cell deficit and increased β-cell apoptosis in T2DM. Though an increased β-cell workload (insulin resistance) is a risk factor for T2DM, most individuals adaptively increase insulin and IAPP expression and secretion without β-cell failure. Experimental evidence supports the concept of a threshold of synthetic burden in β-cells expressing amyloidogenic human IAPP beyond which accumulation of misfolded toxic oligomers comprises β-cell function and viability. We propose that the wide range of β-cell numbers between individuals that becomes apparent after the period of postnatal β-cell mass expansion may serve as an important predictor of risk for T2DM. In those individuals with a relatively low compliment of β-cells, insulin resistance would markedly increase the already high burden for IAPP and insulin folding and disposal (secretion or degradation) per β-cell, potentially exceeding the threshold in at least some β-cells. In those β-cells, the accumulating toxic oligomers would compromise β-cell function and viability, leading to a progressive loss of β-cell function and number. As β-cell function declines in the presence of insulin resistance, hyperglycemia that develops can initially be reversed by an increase in insulin sensitivity (for example, delivery of child after gestational diabetes mellitus or introduction of an exercise regimen [95]) but eventually becomes irreversible if sufficient β-cell mass is lost. We thus postulate that the innate β-cell mass that arises according to intrauterine development and genetic imprinting may be an important predictor of risk for T2DM in the setting of insulin resistance. To rigorously test this postulate it would be necessary to measure β-cell mass in vivo in prospective studies of young adults over many years.

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